

Research Article

Impact of soil-borne inoculum on sheath blight disease development in rice

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Abstract: Sheath blight disease of rice caused by *Rhizoctonia solani* AG-1 IA, has become one of the major diseases in some rice-growing areas in recent years. Primary inoculum density seems to be a major factor in disease outbreak. The aim of the current study was to determine the relationship between the primary inoculum density and type and the disease intensity, grain yield and yield loss. Field experiments were conducted in both years of 2017 and 2018 in Guilan province, Iran. Disease incidence and severity were significantly higher when the highest inoculum densities (mycelial and sclerotial) were tested. When sclerotia were applied as the primary inoculum, disease developed more quickly. Based on the results of the current study, in a temperate lowland rice system in Guilan province, sclerotia floating on the water surface after puddling can be the primary source of inoculum and play a major role in sheath blight epidemics whereas mycelia in plant debris probably lose their viability in winter. These results suggested that control of sheath blight disease in order to prevent sclerotia production and reduce the main disease inoculum can be a promising strategy for suppressing this disease in the rice fields of Guilan province.

Keywords: rice, sheath blight, primary inoculum, viable sclerotia, Guilan province

Introduction

Sheath blight disease of rice, caused by *Rhizoctonia solani* Kuhn AG-1 IA, has become an important disease in all temperate and tropical rice production areas, especially in intensive production system (Otomo, 1989; Savary *et al.*, 1994). Miyake (1910) reported this disease for the first time from Japan and then Reinking (1918) recognized it in the Philippines. It has recently become one of the major rice diseases in most of the rice growing countries of the world (Dasgupta, 1992). High temperature and relative

humidity are conducive to the growth of this fungus. Cultivation of high-tillering and semi dwarf cultivars, dense planting conditions and high rates of nitrogen fertilizer are favorable for development of sheath blight disease (Wu *et al.*, 2012). In lowland rice culture, two independent processes, primary and secondary infection, can be distinguished (Savary *et al.*, 1997). Primary inoculum of rice sheath blight in the tropics is mainly soil-borne and thought to consist of: the sclerotia floating on the water surface after puddling which have long been regarded as the main source of inoculum (Hashiba, 1984; Belmar *et al.*, 1987) and mycelium surviving in crop residues (Mew *et al.*, 1980; Kobayashi *et al.*, 1995), and infested seeds (Okhovvat, 1999; Sivalingam *et al.*, 2006). Furthermore the basidiospores of *Thanatephorus cucumeris* (the

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sexual cycle) are considered to be one of the sources of infection in upland rice (Gangopadhyay and Chakrabarti, 1982; Ou, 1985), which are formed midseason during the booting stage on rice under high temperature and high relative humidity conditions (Hashiba and Kobayashi, 1996), but do not seem to have a significant effect on the epidemiology of the rice sheath blight (Kozaka, 1975). Khosravi *et al.* (2011) reported the occurrence of hymenia and basidiospores from *T. cucumeris* in Mazandaran province, Iran. Savary *et al.* (1997) and Guo *et al.* (2006) explained that the rice sheath blight disease cycle usually starts from overwintered asexual primary inoculum, mainly floating sclerotia present in the soil, irrigation water or stubble or fungal mycelia. Fungal mycelia survive on rice debris, infected weeds and seeds. During cold winter conditions, mycelia in plant debris lose viability after harvest. Seed borne inoculum is known to play a significant role in overwintering and long-distance dispersal of the sheath blight pathogen. Binesh and Torabi (1985) reported the percentage of infested seeds in the Amol 2 (an improved rice cultivar) from 22 to 39% in Mazandaran province and fungal inoculum was still viable on 10-19% of infested seeds eight months after harvest. Secondary infection (polycyclic phase) takes place in the upper part of the canopy by strands of mycelium that are produced on the surface of primary lesions and run on the surface of healthy leaves and sheaths to establish new lesions (Kozaka, 1975; Hashiba, 1984). Kozaka (1961) used the terms “vertical spread” and “horizontal spread” to describe sheath blight epidemics. The first refers to the progress of infection along a tiller, from its base to its upper leaves and it is done by means of fungus mycelia. The second refers to disease spread in the crop, across the tillers and rice plants that provide a physical bridge for the running hyphal strands to progress (Hashiba, 1984; Savary *et al.*, 1995). Primary inoculum of rice sheath blight is variable. In the rice fields of Guilan province, the primary inoculum has not been extensively investigated. Therefore, this study was designed to understand the main type of inoculum, its survival and viability over time

and determine the effect of soil-borne inoculum density on disease development as the most important factors for sheath blight disease development in rice.

Materials and Methods

Crop establishment

To investigate the effect of soil-borne inoculum type and density on disease incidence, severity, grain yield and rice grain loss, experiments were conducted based on a factorial design with three replications at the Rice Research Institute of Iran experimental field (Rasht, Iran) for two years. Nurseries for Shiroudi, a high-yielding and sensitive rice cultivar were established on 20 April 2017 and 16 April 2018. The plot size was 3 × 3 square meters (m²). Seedlings were transplanted at a rate of 3 per hill, with a spacing of 20 × 20 cm between the hills. Fertilizer N (250 kg N/ha) was applied, in two equal parts, as basal incorporation before transplanting and mid-tillering stage.

Sclerotial production and inoculation

An isolate of *Rhizoctonia solani* AG-1 IA (G309) identified by nine pairs of specific primers as a virulent isolate was used for sclerotia production in both years (Padasht-Dehkaei *et al.*, 2012). The sclerotia production was improved using detached leaf inoculation technique (Guleria *et al.*, 2007). Rice leaves from 45-day-old Shiroudi cultivar were cut from the base, washed with distilled water and were placed in sterile plastic petri dishes containing moistened filter paper. Single sclerotia were transferred onto potato dextrose agar (PDA) and were incubated for 48 hours at 27 °C, then 5 mm disks from germinated hyphal tips were transferred to the center of the leaves in the petri dishes. Sclerotia produced after 10 to 14 days were picked and transferred to sterile plastic petri dishes and left to dry at room temperature for 24 h. Sclerotia were counted to prepare 4 sclerotial densities including 0 (control), 12, 24 and 36 sclerotia per 9 m² in the field. The experiment was conducted in a

factorial design with four treatments and three replications.

Mycelium production and inoculation

Sclerotia of *R. solani* produced in previous experiment were transferred on PDA medium slants for 5 days at 28 °C. Rice hull and rice grain mixture (2:1, v/v) were soaked for 24 h and sterilized three times in 500 ml glass bottles. After inoculating grain/hull mixture in the bottles with 48 h growth of the pathogen, they were incubated at room temperature (25-27 °C) in darkness for 15 days. The plots were inoculated after puddling and before transplanting by spreading 0 (control), 30, 60 and 90 g m⁻² of *R. solani*-infested whole rice grain/ rice hull on to the soil surface (Groth and Nowick, 1992).

Disease assessment

Sheath blight incidence was measured as the number of infected tillers relative to the total number of tillers of 25 randomly selected plants from four square meters in each plot. Disease severity of rice sheath blight was estimated based on the relative height of the lesions (RLH) to the plant height. Disease incidence and severity were evaluated at panicle initiation and grain filling in four square meters per plot. Yield losses were determined as below.

$$\text{Yield loss (\%)} = [Y_c - Y_t] / Y_c$$

where, Y_c is the amount of product in control plot and Y_t is the amount of product per each treatment.

Data analysis

Data were subjected to the analysis of variance (SAS, 2003). Means were compared based on the Tukey's test at the 0.05 probability level. Mathematical and statistical analyses were performed through software Statgraphics centurion XVI Version 15.2.05 (StatPoint Inc., Herndon, VA, USA).

Results

The impact of soil-borne inoculum type and density on disease severity and incidence and rice grain yield and yield loss were evaluated in two years. No disease symptom was observed in the uninfected control plots for either mycelial or sclerotial inoculum in the experimental fields. There were no evidences of inoculum motion in floodwater from inoculated to control plots. Initiation and later development of sheath blight disease were variable in different inoculum densities and types.

Variance analysis of disease incidence, severity (Table 1), rice grain yield and yield loss (Table 2) revealed significant differences between different densities of mycelial and sclerotial inoculum ($P < 0.01$) in two years. Mean comparison of disease incidence and severity using tukey's test ($P = 0.05$), divided different densities of the inoculum into four groups (Table 3). Disease incidence and severity were significantly higher where the highest inoculum densities (mycelial and sclerotial) were tested (90 g m⁻² *R. solani*-infested whole rice grain/ rice hull and 36 sclerotia per 9 m²) (Fig. 1).

Table 1 Variance analysis of disease severity and incidence at different inoculum types and densities.

Source of variation	df	Disease incidence				Disease severity			
		Panicle emergence		Grain filling		Panicle emergence		Grain filling	
		MS/2017	MS/2018	MS/2017	MS/2018	MS/2017	MS/2018	MS/2017	MS/2018
Replication	2	0.398 ^{ns}	0.860 ^{ns}	0.605 ^{ns}	0.911 ^{ns}	1.541 ^{ns}	3.291*	0.375 ^{ns}	0.166 ^{ns}
Inoculum density (I)	3	1183.609**	1394.390**	1583.981**	1718.264**	2247.833**	2336.333**	4254.777**	4174.375**
Inoculum type (T)	1	1804.452**	2184.526**	2093.056**	2216.219**	3408.166**	3552.666**	3174.00**	3197.041**
T × I	3	217.457**	266.093**	266.035**	269.263**	415.388**	1309.555**	427.444**	419.263**
Error	14	0.601	1.768	1.341	1.304	1.065	3.529	2.041	1.261
CV		3.912	6.170	5.001	4.765	3.811	6.750	3.969	3.102

* = significant at 5% level, ** = significant at 1% level.

Table 2 Variance analysis of yield and yield loss at different inoculum types and densities.

Source of variation	df	Yield		Yield loss	
		MS/2017	MS/2018	MS/2017	MS/2018
Replication	2	13746.167 ^{ns}	5850.128 ^{ns}	1.740 ^{ns}	1.665 ^{ns}
Inoculum density (I)	3	812704.005 ^{**}	1072986.381 ^{**}	200.098 ^{**}	261.976 ^{**}
Inoculum type (T)	1	119871.302 [*]	388437.518 ^{**}	28.675 [*]	90.777 ^{**}
T × I	3	15966.794 ^{ns}	52852.676 ^{**}	3.943 ^{ns}	12.860 ^{**}
Error	14	16936.779	10865.573	4.396	3.259
CV		2.196	1.780	29.671	21.728

*= significant at 5% level, **= significant at 1% level.

Table 3 Mean comparison of disease severity and incidence at different inoculum densities.

Inoculum density	Means of disease severity				Means of disease incidence			
	Panicle emergence		Grain filling		Panicle emergence		Grain filling	
	2017	2018	2017	2018	2017	2018	2017	2018
Density 4	31.44A	33.77A	35.54A	36.94A	42.83A	43.50A	60.16A	59.50A
Density 3	27.81B	30.68B	33.25B	34.75B	39.00B	40.00B	51.33B	51.33B
Density 2	20.07C	2175C	23.82C	24.17C	26.50C	27.83C	32.50C	34.00C
Density 1	0.00D	0.00D	0.00D	0.00D	0.00D	0.00D	0.00D	0.00D

The means with different letters show significant difference based on the Turkey's test at the level of 0.05.

Different densities of the inoculum were divided into three groups based on mean comparison results of grain yield and yield loss (Table 4). The results showed that rice yield was significantly decreased at the highest sclerotial and mycelial inoculum densities tested (Fig. 2). In 2017, there were no significant difference between 60 and 90 g m⁻² *R. solani*-infested whole rice grain/ rice hull densities and 24 and 36 sclerotia per 9 m², and they belonged to one group (group C).

The data indicated that there was a positive correlation between grain loss and the inoculum density (Table 5). In other words, rice yield loss was significantly increased by high sclerotial and mycelial inoculum densities (Fig. 2). However, there was no significant difference between 60 and 90 g m⁻² *R. solani*-infested whole rice grain/

rice hull densities and 24 and 36 sclerotia per 9 m² in 2017 and they belonged to one group (group A).

Variance analysis of disease incidence and severity revealed a significant difference ($P < 0.01$) between inoculum types (mycelial versus sclerotial) in two years (Table 1). Its validity was confirmed by comparing the results of mean comparison (Table 6). Disease incidence and severity were significantly higher where the sclerotial inoculum was tested. Variance analysis of grain yield and yield loss also revealed a significant difference ($0.01 < P < 0.05$ for 2017 and $P < 0.01$ for 2018, respectively) between inoculum type (mycelial versus sclerotial) in two years (Table 2). Mean comparison of grain yield and yield loss divided sclerotial and mycelial inoculum into two groups (Table 7).

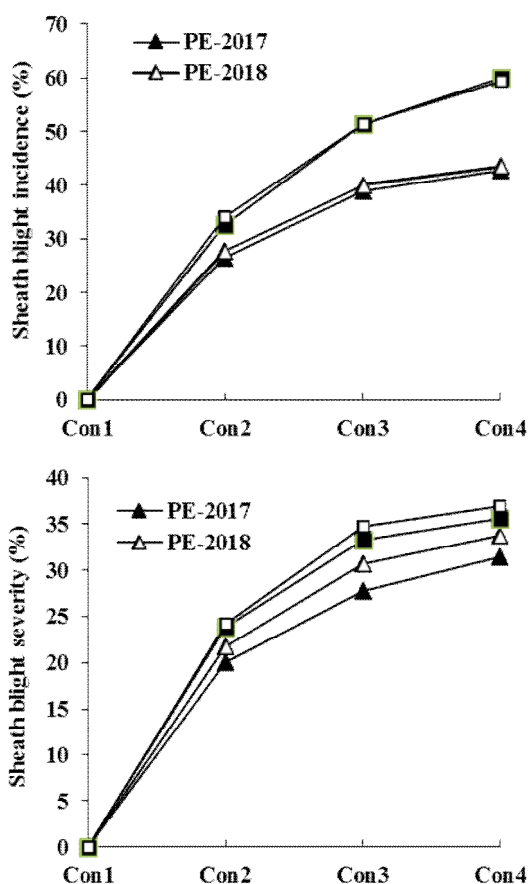


Figure 1 Relationship between disease development and inoculum density. Con1: 0 (control); Con2: 30 g m⁻² *Rhizoctonia solani*-infested whole rice grain/ rice hull/ 12 sclerotia per 9 m²; Con3: 60 g m⁻² *R. solani*-infested whole rice grain/ rice hull/ 24 sclerotia per 9 m²; Con4: 90 g m⁻² *R. solani*-infested whole rice grain/ rice hull/ 36 sclerotia per 9 m². PE: Panicle Emergence. GF: Grain Filling.

Table 4 Mean comparison of yield and yield loss at different inoculum densities.

Inoculum density	Means of yield		Means of yield Loss	
	2017	2018	2017	2018
Density 4	5562.62C	5400.80D	12.75A	15.37A
Density 3	5695.82C	5660.15C	10.66A	11.31B
Density 2	6065.34B	5976.80B	4.86B	6.56C
Density 1	6375.35A	6381.68A	0.00C	0.00D

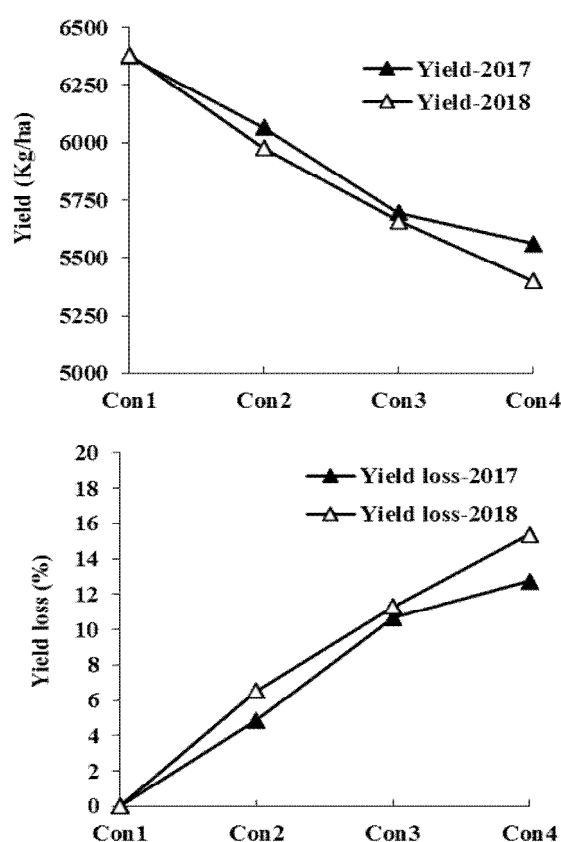


Figure 2 Relationship between grain yield (A) and yield loss (B) in different inoculum densities. Con1: 0 (control); Con2: 30 g m⁻² *Rhizoctonia solani* – infested whole rice grain/ rice hull/ 12 sclerotia per 9 m²; Con3: 60 g m⁻² *R. solani* – infested whole Rice grain/ rice hull/ 24 sclerotia per 9 m²; Con4: 90 g m⁻² *R. solani* – infested whole rice grain/ rice hull/ 36 sclerotia per 9 m².

The data indicated that at the highest density of the sclerotial inoculum, rice yield loss was significantly higher than the highest density for mycelial inoculum and on the contrary, grain yield at the highest density of sclerotial inoculum was lower than the highest density of mycelial inoculum in both years (Fig. 3). According to the observations, sheath blight symptoms occurred two weeks earlier in both years when sclerotia were applied as the primary inoculum and the disease incidence and severity were higher in this situation (Fig. 4).

Table 5 The regression models between different dependent and independent variables based on 2017 and 2018 data.

Year	Dependent Variable	Independent Variable	Model	r	R ²
2017	RLH	SD	RLH = 1.366SD + 7.886	0.92	85.31
2017	INC	SD	INC = 2.183SD + 8.2	0.96	92.93
2017	Y	SD	Y = -26.563SD + 6332.25	-0.95	90.81
2017	YL	SD	YL = 0.416SD + 0.654	0.95	90.83
2017	YL	RLH	YL = 0.275RLH - 0.7	0.93	86.76
2017	YL	INC	YL = 0.183Inc - 0.565	0.95	90.42
2017	RLH	MD	RLH = 0.025MD + 3.603	0.91	82.87
2017	INC	MD	INC = 0.050MD + 4.0	0.96	93.48
2017	Y	MD	Y = -0.899MD + 6359	-0.90	82.44
2017	YL	MD	YL = 0.014MD + 0.260	0.90	82.48
2017	YL	RLH	YL = 0.496RLH - 0.886	0.88	78.42
2017	YL	INC	YL = 0.270Inc - 0.667	0.91	83.38
2018	RLH	SD	RLH = 1.408SD + 8.22	0.92	85.13
2018	INC	SD	INC = 2.15SD + 9.1	0.95	91.59
2018	Y	SD	Y = -33.15SD + 6324	-0.96	94.01
2018	YL	SD	YL = 0.516SD + 0.95	0.96	92.27
2018	YL	RLH	YL = 0.325RLH - 0.688	0.92	85.63
2018	YL	INC	YL = 0.227Inc - 0.62	0.95	90.31
2018	RLH	MD	RLH = 0.027MD + 3.28	0.92	86.11
2018	INC	MD	INC = 0.049MD + 4.56	0.95	92.02
2018	Y	MD	Y = -0.940MD + 6363	-0.97	95.26
2018	YL	MD	YL = 0.014MD + 0.4	0.97	95.18
2018	YL	RLH	YL = 0.480RLH - 0.52	0.93	87.72
2018	YL	INC	YL = 0.279Inc - 0.53	0.95	92.02

r: Correlation coefficient; R²: R-squared; RLH: Disease severity in grain filling stage; INC: Disease incidence in grain filling stage; SD: Sclerotial density; MD: Mycelial density; Y: Yield; YL: Yield loss.

Table 6 Mean comparison of disease severity and incidence at different inoculum types.

Inoculum type	Means of disease severity				Means of disease incidence			
	Panicle emergence		Grain filling		Panicle emergence		Grain filling	
	2017	2018	2017	2018	2017	2018	2017	2018
Sclerotial	28.50A	31.09A	32.49A	33.58A	39.00A	40.00A	47.50A	47.75A
Mycelial	11.16B	12.01B	13.82B	14.36B	15.17B	15.67B	24.50B	24.67B

Table 7 Mean comparison of yield and yield loss in different inoculum types.

Inoculum type	Yield (Kg / ha)		Yield Loss (%)	
	2017	2018	2017	2018
Sclerotial	5854.11B	5727.64B	8.16A	10.25A
Mycelial	5995.45A	5982.08A	5.97B	6.36B

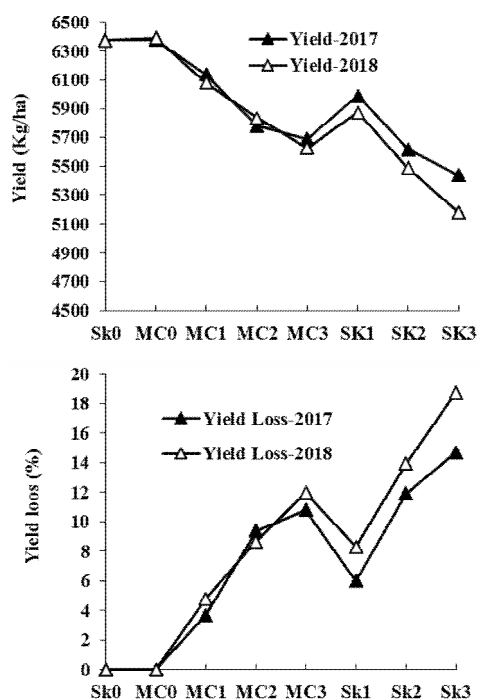


Figure 3 Relationship between rice yield and yield loss and inoculum density and type in 2017 and 2018. Sk0 and MC0: Control; MC1: 30 g m⁻² *Rhizoctonia solani* – infested whole rice grain/ rice hull; MC2: 60 g m⁻² *R. solani* – infested whole rice grain/ rice hull; MC3: 90 g m⁻² *R. solani* – infested whole rice grain/ rice hull; SK1: 12 sclerotia per 9 m²; SK2: 24 sclerotia per 9 m²; Sk3: 36 sclerotia per 9 m².

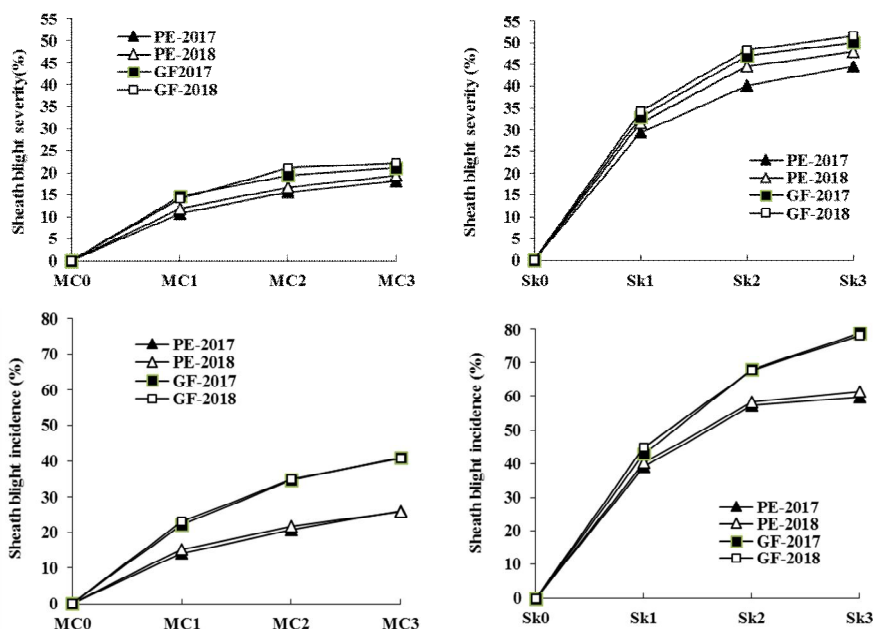


Figure 4 Relationship between disease development and inoculum density and type. Sk0 and MC0: Control; MC1: 30 g m⁻² *Rhizoctonia solani* – infested whole rice grain/ rice hull; MC2: 60 g m⁻² *R. solani* – infested whole rice grain/ rice hull; MC3: 90 g m⁻² *R. solani* – infested whole rice grain/ rice hull; SK1: 12 sclerotia per 9 m²; SK2: 24 sclerotia per 9 m²; Sk3: 36 sclerotia per 9 m². PE: Panicle Emergence. GF: Grain Filling.

Discussion

Sclerotia on the surface of soil or in the soil (Hashiba and Mogi, 1976) and on plant debris, as well as mycelium surviving in plant debris (Mew *et al.*, 1980), and infested seeds (Kozaka, 1970; Damodar Naidu *et al.*, 1983), have been regarded as primary inoculum sources for rice sheath blight. Sclerotia are believed to be the primary source of initial infection in temperate and subtropical rice-growing regions (Hashiba and Mogi, 1975; Lee, 1980; Ou, 1985). Sclerotia although found in tropics, their density in these regions is lower than those reported in temperate and subtropical areas (Lee, 1980; Damicone *et al.*, 1993). In the tropics, mycelia may act as dominant source of initial inoculum. The hypothesis is that the initial inoculum of the pathogen has great impact on disease development. However, determining the relationship between initial inoculum density and disease development for crop diseases which their initial inoculum is soil-borne and cannot be easily measured and counted like rice sheath blight, is difficult.

In this study, we evaluated the effect of *Rhizoctonia solani* soil-borne inoculum type and density on disease development in rice. Mycelial and sclerotial inoculums were applied in different densities in this study. Mycelial inoculum densities were investigated by applying different amounts of *R. solani*-infested whole rice grain/ rice hull (Groth and Nowick, 1992). *R. solani* sclerotia were produced by detached leaf inoculation technique (Guleria *et al.*, 2007). The findings of these trials indicated that disease incidence and severity and grain yield loss were positively and linearly correlated with initial inoculum density of the pathogen. Rice grain yield was negatively and linearly correlated with the initial inoculum density. The sheath blight disease incidence showed more significant correlation with the sclerotial or mycelial inoculum density than the disease severity in both years. Yield loss also showed more significant correlation with the disease incidence than the disease severity in both years (Table 5). Therefore based on the

results of the regression analysis, sclerotial density is a suitable predictor of the disease incidence and the disease incidence is a suitable predictor of yield loss in the field conditions.

The results of the current study were consistent with Tan *et al.* (2007) who reported that disease incidence and severity in the higher inoculation densities increased much more quickly than in the relatively low inoculation densities. In both inoculum type (mycelial or sclerotial), the highest inoculum density showed the highest disease incidence, severity and yield loss. The plots infected with sclerotia in all densities showed a significantly higher disease development than the plots infected with amounts of *R. solani*-infested whole rice grain/ rice hull.

Our finding demonstrated that sheath blight disease in all sclerotial treatments developed much faster and higher than mycelial treatments. These results suggested that sclerotia floating on the water surface after puddling may be the main primary inoculum and play a more important role in sheath blight epidemics. Although the plots that were infected with *R. solani*-infested whole rice grain/ rice hull (as mycelial inoculum) showed sheath blight disease symptom, the disease was significantly lower compared to those plots infected with sclerotia. The findings of this research suggest that in a temperate climate such as Guilan province, mycelia in plant debris lose viability due to the cold winter conditions prevailing after harvest. These results are compatible with findings of earlier works. Kobayashi *et al.* (1997) suggested that though sclerotia are the main primary inoculum of the disease in the Philippines, mycelia in plant debris may also act as initial inoculum in the tropics with short interruption between growing seasons. It has been suggested that sclerotia act as the main primary inoculum in weather conditions of Japan, because in temperate conditions mycelia lose their viability. According to the low sclerotia densities in their sampling, Cu *et al.* (1996) stated that in tropical lowland rice, mycelia of *R. solani* in plant debris probably play a more important role in sheath blight epidemics than sclerotia. However, due to

the difficulty of quantifying, the density of mycelia was not assessed in their study.

Conclusion

In conclusion, the current study demonstrated that not only inoculum density, but also inoculum type (mycelia or sclerotia) were positively correlated with disease incidence, severity and yield loss in 2017 and 2018. Disease development was higher and faster when sclerotia were applied as primary inoculum. In summary, it could be concluded that in Guilan province with temperate climate, sclerotia floating on the water surface after puddling might be the main primary inoculum source and play a more important role in sheath blight epidemics. Mycelia in plant debris probably lose their viability due to the cold winter conditions and are less important in disease development.

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تأثیر مایه‌ی تلقیح خاکزاد بر توسعه بیماری سوختگی غلاف در برنج

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چکیده: بیماری سوختگی غلاف برنج که به‌وسیله قارچ *Rhizoctonia solani* AG-1 IA ایجاد می‌شود، یکی از بیماری‌های مهم در بسیاری از مناطق کشت برنج در سال‌های اخیر می‌باشد. مقدار مایه تلقیح اولیه به‌نظر می‌رسد یک فاکتور مهم در وقوع بیماری باشد. هدف اصلی در این تحقیق، تعیین ارتباط بین مقدار و نوع مایه تلقیح اولیه و مقدار شیوع و شدت بیماری، عملکرد دانه و خسارت محصول بود. آزمایشات مزرعه‌ای در سال‌های ۱۳۹۶ و ۱۳۹۷ در استان گیلان انجام گردید. در بالاترین مقدار مایه تلقیح (میسلیوم و سختینه)، وقوع و شدت بیماری به‌طور معنی‌داری بالاتر بوده است. هم‌چنین در آزمایش استفاده از سختینه به‌عنوان مایه تلقیح اولیه، توسعه بیماری سریع‌تر بوده است. براساس نتایج حاصل از تحقیق حاضر، در استان گیلان با شرایط آب و هوایی معتدل، سختینه‌های شناور روی سطح آب بعد از آماده‌سازی زمین، می‌توانند منبع اولیه و عامل اصلی اپیدمی بیماری سوختگی غلاف باشند و میسلیوم‌های موجود در بقایای گیاهی احتمالاً قدرت زنده‌مانی خود را در سرمای زمستان از دست می‌دهند. نتایج تحقیق حاضر پیشنهاد می‌کند که کنترل بیماری سوختگی غلاف به‌منظور جلوگیری از تولید سختینه و در نتیجه کاهش مایه تلقیح اصلی بیماری، می‌تواند راهکار مناسبی جهت مبارزه با بیماری سوختگی غلاف در استان گیلان باشد.

واژگان کلیدی: برنج، سوختگی غلاف، مایه تلقیح اولیه، اسکروت زنده، استان گیلان